Effects of Extraction and Fractionation Pressures on Supercritical Extraction of Cholesterol from Beef Tallow

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Edible beef tallow was extracted by supercritical CO_2 in a dynamic mode at pressures from 138 to 345 bars and temperatures of 40 and 50°C. The lipid fractions were collected at 34.5 bar/40°C. A retrograde behavior of lipid solubility was observed around 170–175 bar. The ranges of the cholesterol concentration [chol.], were 300–450 mg/100 g and 50–200 mg/100 g lipid for the fractions extracted at 138 bar and 345 bar, respectively. Beef tallow was also extracted with sequentially varied pressures of 138, 345 and 138 bars at 40°C and collected at 34.5 bar/40°C. The results showed that after 20 kg CO_2 was used for extracting 100 g of loaded beef tallow the weight of the residual beef tallow remaining in the extractor was 23 g with [chol.] of 49 mg/100 g lipid. The lower [chol.] of the residual beef tallow represents a 60–70% reduction in cholesterol content, when compared with untreated beef tallow where [chol.] ranges from 130 to 160 mg/100 g lipid. To isolate lipid fractions containing higher [chol.], beef tallow was extracted at 345 bar/40°C and then fractionated into three separators connected in series with decreasing pressures of 173 bar, 117 bar, and 34.5 bar at 40°C, respectively. The results showed that the fractions collected from the third separator (34.5 bar) contained concentrated [chol.] ranging from 272 to 433 mg/100 g lipid. The fatty acid analysis revealed that the fractions containing high [chol.] generally consisted of high concentrations of myristic and palmitoleic acids but low concentrations of stearic and oleic acids.

KEY WORDS: Beef tallow, cholesterol, fractionation, supercritical carbon dioxide.

Use of edible beef tallow (BT) in the food industry usually centers around applications requiring combined lubricity and structure such as cake and icing, shortenings and Danish puff pastry. Beef tallow is also commonly used as a fat medium in commercial frying operations, simply because of "customer preference" due to the unique desirable flavor it adds to fried products, which partially hydrogenated vegetable fats apparently are not able to provide (1,2). Edible usage of BT, however, has been declining gradually over the past several years. This can be attributed mainly to continued health concerns over the impact of animal fat consumption on the development of various diseases. Meanwhile, other researchers have shown that when cholesterol is heated in air it readily oxidizes to form derivatives that might be angiotoxic, cytotoxic or carcinogenic (3–5). Beef tallow (containing 0.15–0.20% cholesterol by weight) under normal frying conditions also forms oxidative products (6). The reduced usage of BT has prompted a renewed interest in developing fractionated BT. Specific fractions with modified cholesterol and/or fatty acid content might be used more satisfactorily as a "tailor-made" edible ingredient, a fat frying medium, an additive for animal feed or other replacements for more expensive imported fats and oils, such as cocoa butter used by the confectionery industry.

A review of published studies has shown that two conventional techniques, dry and solvent fractionation, are usually used to alter the composition of edible fats and oils (7–12). The dry fractionation process is simple and yields clear oil but results in significant olein entrainment in the stearin fraction. The solvent fractionation process provides higher efficiency in filtration and results in better separation in composition between lipid fractions (9,10). However, the equipment used must be flame- and explosion-proof, and further costly downstream processing is needed to fully refine the fractions to remove solvent residue, which may lead to the loss or change of the natural fat flavors.

Development of a process in which supercritical (SC) fluids are used as the extraction medium may allow one to circumvent the drawbacks of the above-mentioned processes. For conventional organic solvent extractions, changes of selectivity of soluble lipid components require changes in solvent composition, whereas SC fluids require only a change in density of the fluid *via* manipulated pressure and temperature to alter the selectivity. Stahl *et al.* (13) have thoroughly reviewed the general physical-chemical properties of typical SC fluids and their wide industrial application in various fields. Other advantages of this extraction process have been described by Tiwari (14) and Chao *et al.* (15,16). Diverse applications of supercritical fluid extraction (SFE) techniques to obtain modified edible oils and fats from natural products have been reported including milk fat (17–21), oilseeds (22–26), color seeds (16), meats (15,27), marine oils (28–33) and rice bran (34,35). As is evident from these studies, SFE is a practical alternative process for the fractionation and separation of fats and oils. The aim of this study was to develop a suitable method with SC-CO_2 to fractionate BT into edible ingredients that may be used for further application to various fabricated foods. Current research effort is focussed on the effects of different SFE operating conditions on the isolation of cholesterol from BT extracts.

EXPERIMENTAL PROCEDURES

SFE unit. The experimental apparatus (Fig. 1) for flow-through SFE was manufactured by Newport Scientific, Inc. (Jessup, MD) and was modified by adding two additional separators. A detailed description of the general operation of the system was presented elsewhere (15), and only a brief summary of the operation is given here for its relevance to the discussion of the results. BT was used as is from commercial-grade crude beef tallow (Anderson Clayton/Humko Products, Inc., Memphis, TN) and was carefully melted, mixed and subdivided into smaller plastic containers with 200 g each in weight, stored at −27°C until used.

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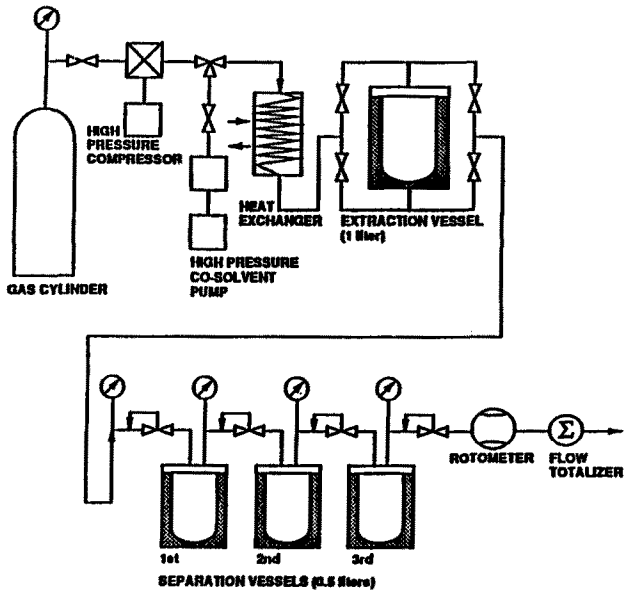

FIG. 1. Supercritical fluid extraction unit with three separators.

For each experimental trial, about 100 g of BT was loaded into a sealed extraction vessel. The pressure and temperature ranges for extractions were 138–345 bar and 40–50°C, respectively. The lipid-loaded SC-CO_2 stream leaving the extractor was then reduced through a back pressure regulator to 34.5 bar/45°C where the dissolved lipid was precipitated and collected in either one or all three separators, depending on the experiments. The pressure for each separator could be individually adjusted to determine the effect of different pressure profiles on the composition of fractionated samples. The flow rate and volume of CO_2 were measured with a flowmeter and a totalizer, respectively. The mass flow rate of CO_2 measured at 20°C was maintained at 3.40–3.75 kg/h, and extraction times ranged from 4–6 h or until total BT was extracted. Extracted samples were collected for each 1.57 kg CO_2 used and were stored in a dark room at −27°C until used for chemical analysis.

Determination of total cholesterol content. The sample preparation for cholesterol content was based on the AOAC method Section 28.110 (36). On a Varian Model 3700 gas chromatograph (GC) equipped with dual flame ionization detectors (Palo Alto, CA), prepared samples were injected to a 30-m × 0.32-mm i.d. Supelco SPB-1 fused-silica capillary column (Bellefonte, PA). The initial holdup time was 4 min at 270°C, and then the temperature was programmed to 300°C at a ramp rate of 10°C/min. Helium flow rate was 1.5 mL/min, and the split ratio was 50:1, while both the injector and detector temperatures were set at 310°C.

Determination of fatty acids. Sample preparation for total fatty acid analysis was modified from AOAC method section 28.056 (36) by changing heptane to hexane. The prepared samples were analyzed in the same model GC unit mentioned above by using a Supelco SP-2330 capillary column with the size of 30 m × 0.32 mm i.d. Both the cholesterol and fatty acids methods were calibrated regularly with standards to maintain the accuracy of GC results at 1%.

RESULTS AND DISCUSSION

SFE with fixed extraction pressures. Figure 2 shows the cumulative yields of the lipid fractions collected in the separator at 34.5 bar/40°C for various operating conditions. The loaded weight of BT for each run was 100 g. For the 345 bar runs, approximately 12 kg CO_2 was needed to extract the loaded BT despite the difference in temperature, while 22 kg CO_2 was needed for the 242 bar/40°C run. A similar amount of needed CO_2 was expected for the 242 bar/50°C run, although extraction of BT was interrupted after 18 kg CO_2 was used. The slopes for the linear portions of the plots (Fig. 2) reveal that the average extraction rate for the 345 bar runs was 11.5 g lipid/kg CO_2 and 5.0 g lipid/kg CO_2 for the 242-bar runs. For the 138-bar runs, however, only around 22% of the loaded BT was extracted after 20 kg CO_2 was used, with the average extraction rate ranging from 0.4–0.8 g lipid/kg CO_2 depending upon the temperature. Therefore, extraction at higher pressures greatly shortened the extraction time with higher yield recovery. Close examination of the temperature effect on lipid extractability revealed that increasing temperature from 40 to 50°C at 138 bar decreased the total lipid yield from 22 to 5%. However, the trend of decreased lipid yield with increased temperature was not found in the 242 bar and 345 bar runs where the lipid yield extracted at 50°C was generally higher than at 40°C. The results indicate that an inversion of lipid solubility occurred at the so-called retrograde extraction pressure of 170–175 bar. Above the retrograde pressure, the lipid extractability of SC-CO_2 increased with extraction temperature, whereas below the retrograde pressure the effect of higher extraction temperature was to decrease lipid extractability. This inversion behavior of lipid solubility in SC-CO_2 has also been reported for soybean, canola, fish oil and lemon oil (37–40). Stahl *et al.* (13) explained that the phenomenon of lipid solubility inversion was caused by the interactions between the density of the SC-CO_2 and the vapor pressures of the weakly volatile components, which are both influenced by the extraction temperature.

The cholesterol concentration [chol.], expressed as mg cholesterol/100 g lipid, for selected extracted fractions for

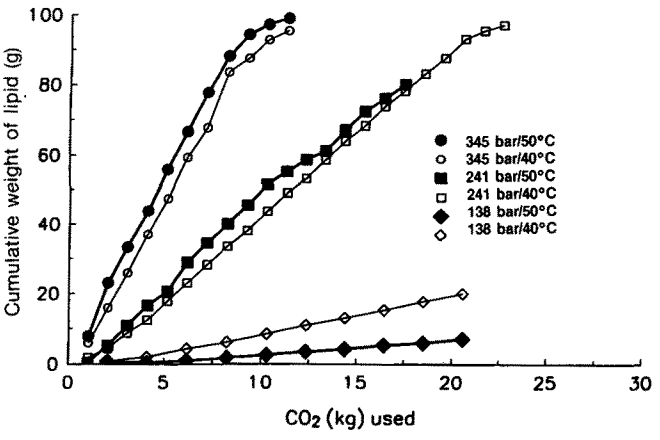

FIG. 2. Cumulative weight of beef tallow extracted by CO_2 with extraction pressure and temperature varied from 138 to 345 bar and 40 to 50°C, respectively.

those runs mentioned above are shown in Figure 3. As expected, the [chol.] of those fractions extracted at 345 bar were the lowest, higher for those fractions extracted at 242 bar, and the highest for those extracted at 138 bar. Hence, extracting BT at lower pressures led to higher selectivity for cholesterol but lower total yield for extracted fat and cholesterol. Apparently, cholesterol has a greater affinity for the lipids extracted at low pressures. However, the current cholesterol analysis can only determine the total cholesterol content and is incapable of differentiating between free dissolved cholesterol, cholesterol esterified to free fatty acids, or cholesterol esterified to the fatty acid moieties of triglycerides. Further research is needed to resolve this relationship.

SFE with sequentially varied extraction pressures. Despite the fact that lipid fractions with high [chol.] can be isolated from BT with lower extraction pressure at 138 bar, the [chol.] of the residual BT from the extractor was around 110 mg/100 g lipid, which was still considered high. On the other hand, extracting BT at high pressures led to rapid extraction and fractionation, but the [chol.] of the fractions was similar to unextracted BT. To obtain considerable reduction in the cholesterol content of the extracted BT, extractions were performed at high and low extraction pressures in sequence. The extractions were run at 40°C by first extracting BT at 138 bar for a certain period of time, then proceeding to extract the remaining BT in the extractor at 345 bar for another time period. Finally, the residual BT was extracted again at 138 bar for a third time period. Two tests, with 100 g and 200 g, respectively, loading weight of BT, were run for the experiment, and 6 kg CO_2 was used for each extraction stage. Figure 4 shows the yields of both lipid and cholesterol, along with the amount of CO_2 used. Yields of extracted lipid and cholesterol were both markedly increased as pressure was changed from 138 to 345 bar at the end of 8 kg CO_2. Similarly, as pressure was shifted back from 345 bar to 138 bar at 14 kg CO_2, the yields of lipid and cholesterol were quickly reduced back to the same level as shown in the first stage of extraction. A similar result of this rapid effect of changing pressure was also reported by Nilssen *et al.* (30) for menhaden fish oil esters. Figure 5 shows the variation of [chol.] for the corresponding fractions described in Figure 4. Both figures reveal that the

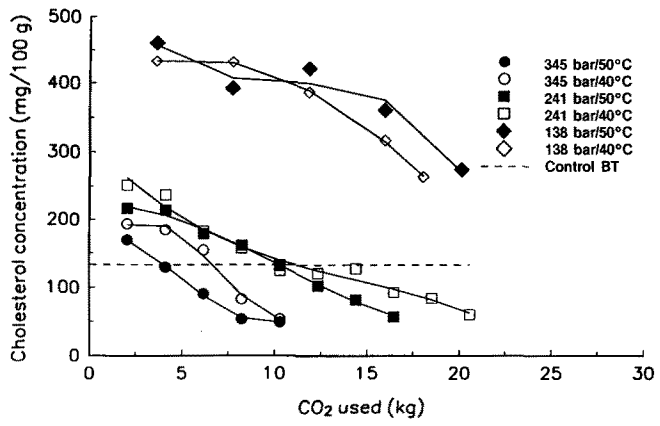

FIG. 3. Cholesterol concentration of selected fractions of beef tallow extracted by CO_2 with extraction pressure and temperature varied from 138 to 345 bar and 40 to 50°C, respectively.

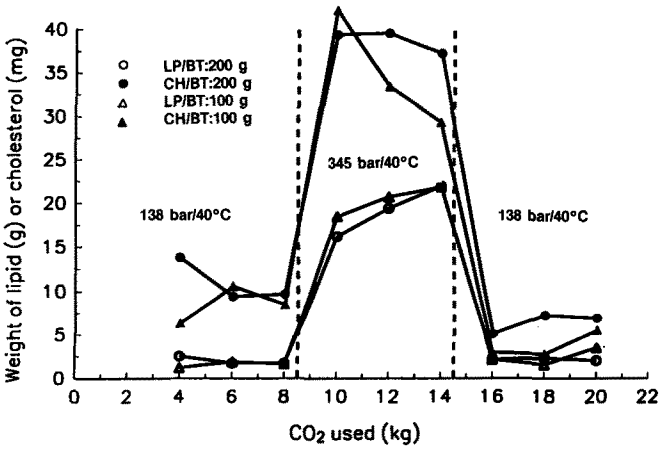

FIG. 4. Weights of lipid (LP) and cholesterol (CH) of beef tallow (BT) fractions extracted by CO_2 with sequentially varied pressure: 138 → 345 → 138 bars at 40°C.

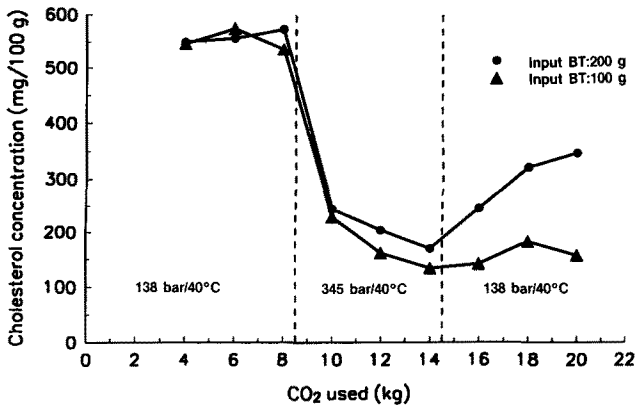

FIG. 5. Cholesterol concentration in different beef tallow (BT) fractions extracted by CO_2 with sequentially varied pressures: 138 → 345 → 138 bars at 40°C.

total yields of lipid and cholesterol obtained from the fractions from each of the three stages of extraction were nearly identical despite the difference in loading weight. The higher yield of cholesterol for the run with 200 g loading weight resulted from the greater amount of cholesterol available for the latter stage of extraction. A material balance for the run with 100 g loading weight showed that after 20 kg CO_2 was used, the weight of BT remaining in the extractor was 23 g with 49 mg/100 g lipid of [chol.]. The reduced [chol.] represents 60–70% of cholesterol reduction when compared with the original BT prior to SFE. However, the lipid yield recovery was also low for this fraction.

Further fractionation of BT with multiple separators. The BT fractions obtained from the above-mentioned runs were collected in only one separator. Because the solvent power of CO_2 depends upon its density, stepwise reduction of separation pressures will alter the density of CO_2, so that the solute-laden CO_2 phase can be further fractionated, provided that two or more separators are used. Table 1 lists the yields of lipid and cholesterol of the selected fractions from BT extracted at 345 bar/40°C and collected from three separators. With temperature preset

TABLE 1

Weights of Lipid and Cholesterol of Selected Beef Tallow (BT) Fractions Extracted at 345 bar/40°C and Fractionated by the Order of 172 bar (S1), 103 bar (S2) and 34.5 bar (S3) at 40°C[a]

CO_2 (kg)	Separator	wt of fraction (g)	[chol.] (mg/100 g)	wt of cholesterol (mg)
6	S1	10.2	128	13.0
	S2	4.1	171	6.9
	S3	7.1	433	30.7
12	S1	11.2	92	10.3
	S2	5.1	126	6.4
	S3	10.3	376	38.8
18	S1	11.9	49	5.8
	S2	4.4	75	3.3
	S3	10.7	272	29.2

[a]Loading weight of BT: 200 g; [chol.] = cholesterol concentration.

at 40°C, the separation pressures of the three separators were adjusted to 170 bar for the first separator (S1), 102 bar for the second separator (S2) and 34.5 bar for the third separator (S3). To accumulate enough sample, fractions from S3 were collected for every 6 kg CO_2 used, while the fractions from S1 and S2 were collected for every 2 kg CO_2 used. With 200 g BT loading weight, the fractions from S3 contained markedly higher [chol.] than the fractions from S2 and S1. Similar results were also found in other repeated runs with 100 g and 200 g loading weights, respectively. When fractions from S1 and S3 were analyzed, the [chol.] obtained was 87 mg/100 g in S1 and 351 mg/100 g in S3. Based on the totals presented in Table 2, a material balance indicated that the total fractions from S3 held 14% of the total lipid weight and 31–38% of the total cholesterol content of BT prior to SFE. Unlike the previous runs in which varying pressures and one separator were used and where high [chol.] was achieved at the expense of low cholesterol yield at low extraction pressure (138 bar), the yield of total extractable cholesterol (mg) of this extraction procedure was high. Thus, extraction at the highest possible pressure with subsequent fractionation in multiple separators is the preferred method of separating BT into high and low cholesterol fractions. Further, while the extracted lipid weights were fairly constant for the fractions from S1 and S2, the [chol.] was, however, largely decreased. Apparently, more cholesterol was carried over by SC-CO_2 to S3 than to S1 and S2. This is further evidence that the cholesterol in BT is somewhat associated with the lipids most soluble in SC-CO_2.

Because the solute-laden CO_2 phase leaving from the extractor can be further fractionated by the multiple separators, it is necessary to determine the effect of the adjustment of separation pressure on the fatty acid composition of the lipid fractions from each separator. Table 2 lists the composition of fatty acids for three fractions collected from each separator at 6 (F6), 12 (F12) and 18 (F18) kg CO_2. For each fraction number, the concentrations of myristic acid, [C14:0], and palmitoleic acid, [C16:1], were consistently increased, whereas the concentrations of stearic acid, [C18:0], and oleic acid, [C18:1], were decreased in order from S1 to S3. Meanwhile, the concentrations of palmitic acid, [C16:0], linoleic acid, [C18:2] and linolenic acid, [C18:3], were fairly constant. When the fractions were compared for each separator, [C14:0] and [C16:1] were decreased whereas [C18:0] and [C18:1] were increased. The trend indicates that as extraction proceeded, with fractionation *via* the three separators, the fractions consisting of triglycerides with more fatty acid moieties of C18:0 and C18:1 and less C14:0 and C16:1 were concentrated in S1. In the meantime, those triglycerides containing higher C14:0 and C16:1, but less C18:0 and C18:1 were carried through S1 and S2 by SC-CO_2 and eventually precipitated in S3. Relating this to the result of markedly high [chol.] for the fractions in S3, it appears that [chol.] in BT is somewhat more associated with C14:0 and C16:1 than homologous C18 fatty acids.

With easy manipulation of extraction and separation conditions, small quantities of lipid fractions with markedly enriched cholesterol content could be separated from the original BT. Technically, high pressures coupled with gradual reduction of the density of SC-CO_2 during separation are needed to achieve good fractionation, while minimizing the process time and quantity of CO_2 used.

TABLE 2

Total Fatty Acid Distribution of Selected Fractions of Beef Tallow Extracted by CO_2 at 345 Bar and 40°C Using Three Separators[a]

Fraction number	Separator	Fatty acid composition (%)						
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
F6	S1	3.2	23.0	3.1	16.2	36.4	2.5	0.3
	S2	4.1	24.4	3.6	14.2	35.1	2.5	0.3
	S3	4.8	22.1	3.8	13.8	33.4	2.5	0.3
F12	S1	2.7	22.3	2.8	17.6	36.7	2.5	0.3
	S2	3.5	23.6	3.3	15.2	35.4	2.6	0.3
	S3	4.8	23.4	3.7	11.4	31.0	2.4	0.3
F18	S1	2.1	21.3	2.5	21.1	38.8	2.6	0.3
	S2	3.0	24.1	3.1	18.4	39.2	2.7	0.4
	S3	4.2	23.3	3.4	12.9	32.4	2.6	0.3
Control		3.3	22.5	3.1	14.3	40.4	3.5	0.5

[a]Loading weight: 200 g.

Extraction with sequentially varied pressures and one separator can also yield extracted BT with substantially low [chol.]. This process method is simpler than multiple separators, but it uses more CO_2. The selectivity of extractable lipid components by SC-CO_2 can be tuned further by the effect of retrograde behavior on the lipid solubility. Research is now being conducted to determine the effect of various adjustments of separation pressures on the separation of cholesterol from those lipid components responsible for the unique flavors of BT. Also, it appears that cholesterol-free fractions are not obtainable by simply adjusting the SC-CO_2 density, and further work is being conducted on the use of in-line adsorbents as part of the SFE process to further lower the cholesterol contents of extracted BT fractions.

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